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Natural *Brucella* Infection in Argentine Wild Foxes*

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In the course of an investigation in 1962-64 into the natural occurrence of brucellosis among grey foxes in Argentina, agglutination tests were performed on 728 sera of the foxes Dusicyon gymnocercus antiquus *and* D. griseus griseus, *captured in the provinces of Buenos Aires and Rio Negro. Agglutination titres of from 1:25 to 1:800 were found in 173 (23.8%) of the foxes tested, 11.3% having titres of 1:100 or more. In bacteriological testing, eight cultures of* Brucella abortus, *biotype 1, were obtained.*

Discussing their findings, the authors point out that it cannot be stated definitely whether Brucella *is naturally shed by foxes or to what extent infected foxes contribute to the dissemination of brucellosis.*

Foxes are common predators in many areas of Argentina. The purpose of the study reported here was to investigate the natural occurrence of brucellosis in the grey foxes of the Pampa and North Patagonia regions.

MATERIALS AND METHODS

Animals

A total of 752 foxes were captured, 434 in the central part of the province of Buenos Aires (Azul and Olavarria Districts) and 318 in the southernmost region of the same province, as well as in the adjacent areas of the province of Rio Negro. The foxes captured in the first region (the Pampa) were identified as *Dusicyon gymnocercus antiquus*, and those of the second region (North-Patagonia) as *Dusicyon griseus griseus*.[3]

The foxes were trapped alive during the years 1962-64 (using Victor No. 2 traps [4]) with a view to studying the presence of zoonotic diseases in them. Blood samples were taken by cardiac puncture, and the animals sacrified by injecting air into the heart. The foxes that were examined bacteriologically were brought to the laboratory, where specimens of different organs were removed aseptically.

Serological tests

The sera of 728 foxes were examined by the plate agglutination test, starting with a 1 : 25 dilution. Any serum that gave a reaction to this dilution was further tested to the end-point by the plate and tube agglutination tests. In this paper the titres are expressed in terms of International Units of Anti-*Brucella abortus* Serum (WHO Expert Committee on Biological Standardization, 1954) and correspond to the results obtained by tube agglutination, with the exception of 17 haemolysed sera that were read only by the plate test.

Bacteriological tests

Bacteriological examinations were performed on 111 specimens of *D. gymnocercus antiquus* trapped on four cattle ranches of the central area of the province of Buenos Aires. No selection was made of animals with respect to their serological status.

Bacteriological tests were first made on 31 pools of organs from 77 foxes. Lymph-nodes (internal iliac, mandibular and suprapharyngeal), spleen and liver tissue from two to four foxes were mixed and ground in a mortar with sterile sand; the tissue suspension obtained was inoculated into blood agar and *Brucella* agar (Albimi Laboratories). In the second phase of the investigation, cultures were made with pools of organs from 34 foxes. After grinding, the tissue suspension was inoculated on modified Kuzdas-Morse medium (Renoux, 1954).

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TABLE 1
RESULTS OF BRUCELLOSIS SERUM-AGGLUTINATION TESTS IN FOXES OF THE PAMPA AND NORTH PATAGONIA REGIONS, ARGENTINA, 1962-64

Origin	Number of samples examined	Titre							Percentage with titres of 1:25 to 1:800	Percentage with titres of 1:100 or more	Cattle	
		Neg.	1:25	1:50	1:100	1:200	1:400	1:800			Number examined	Percentage reacting with titre of 1:100 or more
Pampa region:												
Ranch 1	86	64	2	5	4	10	1	0	25.6	17.4	625	11.4
Ranch 2	29	16	3	3	4	3	0	0	44.8	24.1	1 050	12.3
Ranch 3	49	41	2	2	2	2	0	0	16.3	8.2	—	—
Ranch 4	31	29	1	0	1	0	0	0	6.5	3.2	1 308	32.4
Ranch 5	118	96	7	4	4	4	1	2	18.6	9.3	2 214	6.3
Ranch 6	26	16	1	5	2	1	1	0	38.5	15.4	1 342	6.5
Ranch 7	62	37	7	4	5	4	2	3	40.3	22.6	—	—
Ranches (several)	9	7	0	1	0	1	0	0	22.2	11.1	—	—
Total	410	306	23	24	22	25	5	5	25.4	13.9		
North Patagonia region:												
Ranch 1	208	165	12	19	10	2	0	0	20.7	5.8		
Several [a]	110	84	1	12	8	5	0	0	23.6	11.8		
Total	318	249	13	31	18	7	0	0	21.7	7.9		
Grand total	728	555	36	55	40	32	5	5	23.8	11.3		

[a] Comprises two different regions in the south of Buenos Aires Province and the south-east of Rio Negro Province.

The inoculated Petri dish media were incubated for a week at 37°C in atmosphere containing 10% CO_2. Typical *Brucella* colonies were transferred and the cultures identified by the conventional methods: microscopic examination of Gram-stained preparations, agglutination with specific serum, motility testing, and determination of fermentation ability of carbohydrates.

The *Brucella* isolates were typed by the conventional biochemical and serological methods. Phage susceptibility was determined with a preparation of the phage Tb (Tbilisi) at 1 RTD (routine test dilution), according to the techniques described by Morgan (1963). The urease tests were done by the method of Stuart et al. (1945). The monospecific sera were produced according to the techniques described by Jones (1958). All tests were performed in comparison with the FAO/WHO *Brucella* reference strains (Joint FAO/WHO Expert Committee on Brucellosis, 1952). Oxidative metabolic tests were performed by Dr Margaret E. Meyer, at the School of Veterinary Medicine, Davis, Calif., USA.

The strains were further tested for sensitivity to thionin blue at 1 : 500 000 concentration and to 5 IU and 10 IU of penicillin per ml.[1]

RESULTS

Serological tests

The results of the agglutination test are shown in Table 1. Of the 728 fox sera examined, 173 (23.8%) showed titres ranging from 1 : 25 to 1 : 800, and 82 (11.3%) of these had titres of 1:100 or more.

In the Pampa region, where the fox is *D. gymnocercus antiquus*, 25.4% of the 410 animals examined had detectable agglutination titres, 13.9% of these being of 1 : 100 or more. When the figures are analysed by place of capture (i.e., the ranch on which the foxes were trapped), the rate of reactors at any titre (1 : 25 or more) varied from 6.5% to 44.8% and that of reactors at a titre of 1 : 100 or more from 3.2% to 24.1% respectively. On some of the ranches

[1] Morgan, W.T.B. (1963) unpublished document WHO/Bruc/247.

TABLE 2
RESULTS OF BRUCELLOSIS AGGLUTINATION TEST IN FOXES ACCORDING TO SEX

Sex	Total examined	Titre							Percentage with titres of 1:25 to 1:800	Percentage with titres of 1:100 or more
		Neg.	1:25	1:50	1:100	1:200	1:400	1:800		
Males	353	275	15	27	18	14	2	2	22.1	10.2
Females	364	274	19	27	20	18	3	3	24.7	12.1
Unclassified	11	6	2	1	2	0	0	0	—	—
Total	728	555	36	55	40	32	5	5	23.8	11.3

however, the number of foxes captured was too small for these figures to be significant. It is noteworthy that, in four of the five ranches where the agglutination test was performed in cattle as well, the percentage of foxes reacting (at 1 : 100 or more) was higher than that of cattle (Table 1).

In the North Patagonia region, where the fox is *D. griseus griseus*, the percentage of reactions at 1 : 100 or more was lower than in the Pampa region (7.9% as against 13.9%), but there was no significant difference when the full range of titres (1 : 25 to 1 : 800) is considered.

As shown in Table 2, about the same numbers of males and females were tested. There was no significant difference in the rate of reactions by sex.

Bacteriological tests

Brucella cultures were obtained from 5 (16.1%) of the 31 pools of organs from 77 foxes (*D. gymnocercus antiquus*). As was shown by agglutination tests performed later, four of these five bacteriologically positive pools contained organs of one or two individuals with a titre of 1 : 100 or higher. The fifth positive pool was of the organs of two dead foxes that were not tested serologically.

Among the 34 individually examined foxes of the same species, the pools of lymph-nodes, spleen, liver and genital organs of three were found bacteriologically positive. These three animals (one male and two females) had agglutination titres of 1 : 25 (1 : 50 in the plate test), 1 : 100 and 1 : 200 respectively. Of the 31 bacteriologically negative foxes, 15 were dead on arrival at the laboratory and were not tested serologically, two had titres of 1 : 25, and 14 were serologically negative.

All eight cultures (five from 31 pools of 77 foxes and three from individual foxes) were typed. All isolates required CO_2 for their initial growth. Their behaviour on thionin- and fuchsin-containing media (dilutions 1 : 25 000, 1 : 50 000, 1 : 75 000 and 1 : 100 000) were similar to that of the *Br. abortus* 544 reference strain. These strains had a more vigorous hydrogen sulfide production and urease activity than the reference strain, but were similar in this respect to *Br. abortus* strains isolated from Argentine cattle. All isolates agglutinated with *Br. abortus* monospecific serum but not with *Br. melitensis*; all were lysed by the Tb phage at 1 RTD. On the basis of these tests the strains were classified as *Br. abortus*, biotype 1. The typing was confirmed by Dr Margaret E. Meyer, Davis, Calif., who also conducted oxidative metabolic tests with these strains.

The fox isolates were not inhibited by thionin blue and penicillin at the concentrations tested, showing in this respect the same behaviour as other *Br. abortus* field strains.

DISCUSSION

The serological study performed shows a high prevalence of brucellosis in grey foxes, both in the Pampa region and in North Patagonia. The isolation of *Brucella* from the foxes leaves no doubt about the origin of the agglutination reactions, but no conclusions can be drawn as to the significance of the different titres. Even if only titres of 1 : 100 or more are taken into consideration, a rather high percentage (11.3%) of foxes may be considered probably infected. As to the prevalence of infection according to sex, the results obtained in this study (Table 2) do not agree with the observations of Rementzova (1962) that female foxes (*Vulpes vulpes*) are more likely to contract brucellosis than males. However, the difference in the fox species affected may account for this difference in results.

The role of foxes and other wild species in the epizootiology of brucellosis is still a subject of controversy. Many wild vertebrates are known today to be naturally or artificially susceptible to *Brucella* infection (Rementzova, 1962; Renoux, 1957; Renoux, 1966). Though the majority of research workers agree that the European hare plays an important role as a natural reservoir of brucellosis (Joint FAO/WHO Expert Committee on Brucellosis, 1958), opinions are divided as to the importance of other wild animal species in the dissemination and maintenance of *Brucella* infection.

The occurrence of brucellosis in European foxes (*Vulpes vulpes*) in their natural habitat has been recognized in Bulgaria. Pavlov et al. (1960) examined serologically 440 red foxes (only 21 of them adults), and found 16 with positive and six with suspicious titres. A strain of *Br. suis* was isolated by them from one of the foxes. The susceptibility of this animal species to *Brucella* infection has also been substantiated on several occasions in Poland and in the USSR (Rementzova, 1962) among fox colonies fed with infected domestic-animal viscera, meat and foetuses. Rementzova (1962) isolated six *Brucella* strains from two fox (*V. vulpes*) colonies in the USSR.

The fact that Pavlov et al. (1960) isolated *Br. suis* from a fox in an area of Bulgaria where swine brucellosis is prevalent and that *Br. abortus* has been isolated in Argentina in regions with a high prevalence of bovine brucellosis might be an indication that the infection is transmitted from domestic animals to the foxes. The finding by the Bulgarian authors of swine foetuses and placentas in the stomachs and intestines of foxes might explain the mechanism of the transmission.

If we accept that fox brucellosis is an extension of the infection from domestic animals, the question remains whether the infection may survive independently in wild foxes and to what degree it may be retransmitted to domestic species. That infection in foxes is long-lasting has been shown by Rementzova (1962), who, examining 29 animals a year after they had been fed an infected meal, isolated *Brucella* from one and found seven serological reactors. Another important fact in this connexion is the occurrence of abortion in infected foxes (Rementzova, 1962). Our present knowledge, however, does not permit us to state definitely either whether *Brucella* is naturally shed by foxes or to what extent infected foxes may contribute to intra- and inter-species spread of brucellosis.

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RÉSUMÉ

Le rôle des renards et d'autres espèces sauvages dans la transmission de la brucellose est encore discuté. On connaît de nombreux vertébrés sauvages susceptibles d'être infectés naturellement ou expérimentalement. Les auteurs ont étudié, par des méthodes sérologiques et bactériologiques, l'existence de la brucellose chez des renards sauvages d'Argentine. Sur 728 animaux examinés, 410 *Dusicyon gymnocercus antiquus* et 318 *Dusicyon griseus griseus* capturés dans les provinces de Buenos Aires et du Rio Negro, des anticorps agglutinants ont été trouvés chez 13,9% des *D. gymnocercus* et 7,9% des *D. griseus*. Le nombre des mâles et celui des femelles étaient à peu près égaux et les taux de positivité suivant le sexe n'ont pas montré de différences significatives, contrairement à ce qui a été observé en Europe chez *Vulpes vulpes* dont la femelle paraît contracter la brucellose plus facilement que le mâle. Dans plusieurs fermes, les résultats des épreuves d'agglutination chez le renard ont été comparés à ceux trouvés chez les bœufs; le pourcentage des renards ayant un titre de 100 UI ou plus a été supérieur à celui des bœufs.

Des examens bactériologiques ont été pratiqués sur 34 *D. gymnocercus* et sur 31 pools d'organes provenant de 77 renards de la même espèce; des souches de *Brucella* ont été isolées de 5 pools et de 3 prélèvements individuels. Quatre des cinq pools bactériologiquement positifs contenaient des organes d'un ou deux renards ayant des titres de 100 UI ou plus. Les deux renards du cinquième pool n'avaient pas subi d'examen sérologique. Les huit souches isolées ont été identifiées comme *Br. abortus* biotype 1.

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